RAPID COMMUNICATION

Association of MMP-8 polymorphisms with tendinopathy of the primary posterior tibial tendon: a pilot study

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INTRODUCTION

Tendon disorders are common and are treated on a daily basis in orthopedic foot and ankle practices. Some tendons are particularly vulnerable to primary degenerative changes, such as the patellar, Achilles, rotator cuff, biceps, posterior tibial, and fibular tendons.[1]

Significant advances in histopathology and research imaging techniques have contributed to an improved understanding of the pathophysiology of tendon degeneration.[2] However, both mechanical factors and vascular and neurological disorders have limitations in explaining the etiology of many cases.[1,3,4] It is known that intense physical activity may predispose an individual to tendinopathy, but some individuals have a predisposition with no clinically recognized cause.

The literature suggests that individual characteristics, including genetic inheritance, may influence the likelihood of developing tendinopathy. Thus, there is a group of individuals with a genetic background that causes increased susceptibility to diseases of the tendon.

Matrix metalloproteinases (MMPs) are a pivotal family of zinc-dependent enzymes responsible for the degradation of extracellular matrix components, including basement membrane collagen, interstitial collagen, fibronectin and various proteoglycans, during normal remodeling, repair processes, development and inflammation. MMPs are expressed in response to specific stimuli by resident connective tissue cells and by the major inflammatory cell types that invade tissues during remodeling events, including tendinopathy.

MMP-8, or collagenase-2, was initially discovered in neutrophils, in which it was thought to be exclusively produced, but this enzyme was subsequently shown to be expressed by a variety of other cell types, including endothelial cells, smooth muscle cells, macrophages, polymorphonuclear leukocytes, gingival fibroblasts, keratinocytes, chondrocytes, odontoblasts[5-10] and oral[11] cancer cells.

It has been suggested that MMP-8 degrades type I collagen, thereby contributing to tissue degradation and remodeling.[12] MMP-8 is an important mediator of tissue destruction in several inflammatory diseases and is related to cardiovascular disease,[9] bronchiectasis,[13] pulmonary insufficiency,[14] periodontitis,[15-17] melanoma,[18] cancer of the head and neck,[19] and diabetic wound healing.[20]

The MMP-8 gene, located on chromosome 11, contains functional polymorphisms in the promoter region, including the substitution of a cytosine by a thymine at position -799 (rs11225395).[21] Alterations in this gene have been associated with chronic dilatation of the bronchi13 and breast cancer.[22]

The discovery of genetic markers of tendinopathy risk could allow for the identification of susceptible individuals and, thus, early therapeutic interventions. MMPs play key roles in tissue destruction and may have important roles in the pathogenesis of tendinopathy. Therefore, we hypothesized that the -799C/T polymorphism in MMP-8 was associated with tendinopathy of the primary posterior tibial tendon and could be a risk factor for this condition.

MATERIALS AND METHODS

Subject selection

A total of 64 subjects were recruited from the patient pool at the Foot and Ankle Group, Department of Orthopedics, at the Traumatology Hospital of the University of São Paulo, Brazil. After a brief explanation of the study, as recommended by the the hospital's Research Ethics Committee, subjects were identified according to age, sex, diagnosis, pathology, medical history, postoperative complications, medication use, personal history of systemic diseases, and family history of infectious and inflammatory diseases. The subjects were divided into two groups, with 66% female and a mean age of 51 years:

Test group - 14 patients undergoing surgical procedures who had pathological diagnoses of degenerative lesions of the posterior tibial tendon.

Control group - 50 patients with posterior tibial tendon integrity and with no signs of degeneration based on magnetic resonance images produced to investigate complaints about the ankle and foot.

All the subjects were in good general health and did not display any of the following exclusion criteria: rheumatic diseases, immunological diseases, diabetes, hepatitis or prior or current infections in the topography of the foot and ankle.

DNA sampling and extraction

Buccal epithelial cells were sampled as described by Trevilatto and Line (2000),[23] and DNA extraction was performed as by Aidar and Line (2007).[24] The DNA concentration was estimated by measuring the absorbance ratio at 260/280 nm.

Polymerase chain reaction and restriction endonuclease digestion

The MMP-8 genotypes were determined using the polymerase chain reaction - restricted fragment length polymorphism (PCR-RFLP) assay. The PCR primers used for amplifying the MMP-8 polymorphism were the forward primer 5′-CAGAGACTCAAGTGGGA-3′ and the reverse primer 5′-TTTCATTTGTGGAGGGGC-3′. The PCR reactions were performed in a total volume of 10 μl that contained 400 ng of genomic DNA, 5 μl of SYBR GREEN JUMPSTART TAQ READY MIX (Amersham Pharmacia-Biotech, Uppsala, Sweden) and 200 nmol of each primer. A 6-μl aliquot of each PCR product was then digested with 1 unit of SfcI enzyme at 37℃ overnight.

Gel electrophoresis

The entire digest was electrophoresed on a 10% vertical non-denaturing polyacrylamide gel at 20 mA. The gel was stained with ethidium bromide.

Statistical analysis

The significance of the differences in the observed frequencies of polymorphisms between both groups was assessed using the chi-squared test, with a $p<0.05$ indicating statistical significance.

RESULTS

The primers used for PCR efficiently amplified the MMP-8 locus of interest, and the *Sfc*I enzyme digestion cleaved the PCR products into two fragments when the polymorphic site contained the C allele (but not T). Electrophoresis produced DNA bands of 32 and 74 bp for C alleles and a band of 106 bp for T alleles, whereas the heterozygote displayed a combination of both alleles (32, 74, and 106 bp).

There was a significant difference in the allele frequencies of the control and test groups ($p = 0.0006$). In the control group, the C allele was observed with a frequency of 71%, whereas in the test group, the T allele was present at a frequency of 64.3%. The C/C genotype was found in 66% of the control group, whereas the T/T genotype was observed in 50% of the test group ($p = 0.0036$). The frequencies of the different alleles and genotypes of the MMP-8 gene are shown in Table 1.

Table 1 - The distribution of the MMP-8 alleles and genotypes in the control and test groups.

MMP-8	Control Group		Test Group		*p*-value
	n	%	n	%	(chi-squared)
Allele	n = 100		n = 28		
C	71	71.0	10	35.7	$p = 0.0006$
T	29	29.0	18	64.3	
Genotype	n = 50		n = 14		(Fisher's exact test)
C/C	33	66.0	03	21.4	
T/T	12	24.0	07	50.0	p = 0.0036
C/T	05	10.0	04	28.6	

DISCUSSION

Posterior tibial tendon dysfunction has been associated with obesity, hypertension, and diabetes.[25] There has also been an association with decreased vascular supply.[26,27] However, some patients have a predisposition for this condition without a clinically recognized cause, which suggests that genetic inheritance may play an important role in tendinopathy.

Some studies have already shown the influence of genetic polymorphisms on tendinopathy. Mokone and collaborators[28] demonstrated that a polymorphism in the alpha 1 type V collagen gene (COL5A1) was associated with Achilles tendon pathologies, with the A2 allele appearing to play a protective role. The same group also demonstrated the role of tenascin-C in Achilles tendon pathologies.[29] By analyzing a guanine-thymine (GT)n repeat polymorphism in the tenascin-C gene, the authors showed that individuals with 12 or 14 GT repeats had a 6-fold higher risk of developing lesions in the Achilles tendon. A G/T polymorphism in the alpha-1 chain of type I collagen has been associated with anterior cruciate ligament rupture[30] but not with Achilles tendon injuries.[31] Raleigh and collaborators[32] found that variants within the MMP-3 gene were associated with Achilles tendinopathy but not Achilles tendon rupture. The presence of cholesterol deposits in the tendons (tendon xanthomas) of familial hypercholesterolemia patients has been associated with polymorphisms in the reverse cholesterol transport and low-density lipoprotein oxidation pathways.[33]

In the present pilot study, the -799C/T polymorphism in the promoter region of the MMP-8 gene was associated with tendinopathy of the primary posterior tibial tendon. The C allele was observed in the majority of the control group, whereas the T allele was more frequent in the test group. Patients bearing the T allele were more likely to have lesions in the posterior tibial tendon. It is possible that this allele can provide the molecular basis for a more intense degradation of the extracellular matrix, which could lead to an increased susceptibility to lesions in the posterior tibial tendon. The clinical significance of these results, however, must be interpreted with caution because the data were derived from only 64 patients (including only 14 in the test group), which provided a moderate power to detect a statistical relationship between the polymorphism and the disease.

Indeed, genetic polymorphisms probably influence the degeneration of tendons through the cumulative effect of multiple polymorphisms that involve complex interactions between multiple genes.[34] Understanding the importance of each polymorphic allele is necessary for assessing the contribution of each polymorphism to the disease phenotype.[35]

Further investigations should determine the roles of several genetic polymorphisms in maintaining homeostasis of the tendon and in tendon pathologies. Because MMP-8 affects the degradation of a large number of extracellular proteins and influences the degradation and remodeling of injured tissues, the study of this gene is important for a better understanding of the process of tendon degeneration. The determination of the genetic patterns of patients with tendinopathy could enable the identification of individuals at higher risk of developing this disease and those with

impaired regeneration capabilities. Thus, any identified genetic markers may contribute to the appropriate preoperative selection, the preparation of strategies for prevention, and individualized therapy to modulate the effects of the genetic markers and increase the success rate of treatments. Thus, it is important that a larger test group be studied in the future to more conclusively identify the influence of the -799C/T MMP-8 polymorphism on the disease phenotype.

CONCLUSION

The utilized primers were effective for the PCR-RFLP analysis of the -799C/T polymorphism in the MMP-8 gene. The preliminary results indicate that this polymorphism may be a risk factor for tendinopathy and could be used as a genetic marker for the primary lesions of the posterior tibial tendon.

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